

***Prokr2-P2A-iCre* Cas9-KI Strategy**

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Reviewer

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Project Overview

Project Name

Prokr2-P2A-iCre

Project type

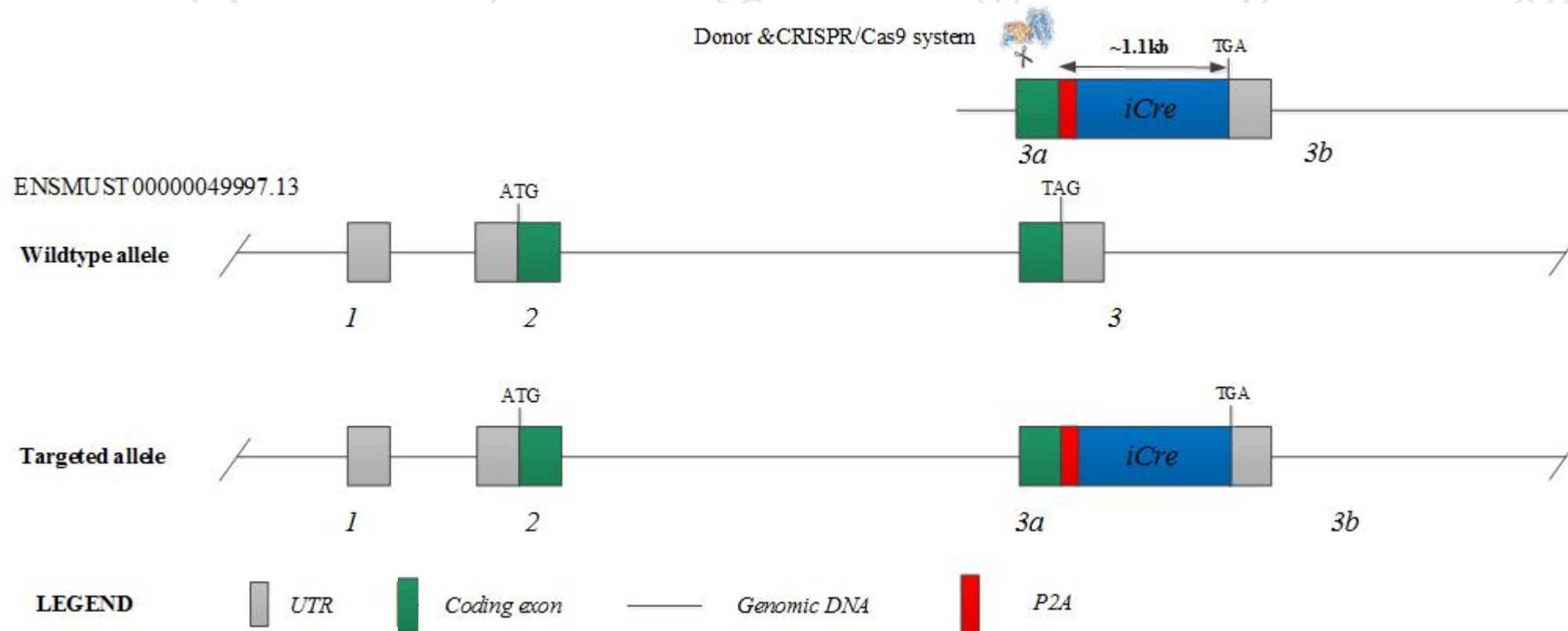
Cas9-KI

Strain background

C57BL/6J

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Prokr2* gene. The schematic diagram is as follows:



- The *Prokr2* gene has 5 transcripts. According to the structure of *Prokr2* gene, *Prokr2-201*(ENSMUST00000049997.13) is selected for presentation of the recommended strategy.
- *Prokr2-201* gene has 3 exons, with the ATG start codon in exon2 and TAG stop codon in exon3.
- We make *Prokr2-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop coding(TAG) of *Prokr2* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre before stop coding(TAG) of *Prokr2* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- According to the existing MGI data, Homozygotes for a null allele show 50% neonatal lethality, olfactory bulb malformation, and reproductive system atrophy related to a lack of hypothalamic gonadotropin-releasing hormone synthesizing neurons. Homozygotes for another null allele show impaired circadian behavior and thermoregulation.
- According to the existing JAX data, In females, expression of Prokr2-Cre is found in the medial preoptic area, ventromedial nucleus of the hypothalamus, arcuate nucleus, medial amygdala and lateral prebrachial nucleus. In males, expression occurs in the amygdalo-hippocampal area.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of iCre may affect the regulation of the 3' end of the *Prokr2* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon3 of *Prokr2* gene in this strategy.
- The *Prokr2* gene is located on the Chr2. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Prokr2 prokineticin receptor 2 [*Mus musculus* (house mouse)]

Gene ID: 246313, updated on 12-Aug-2019

 Summary

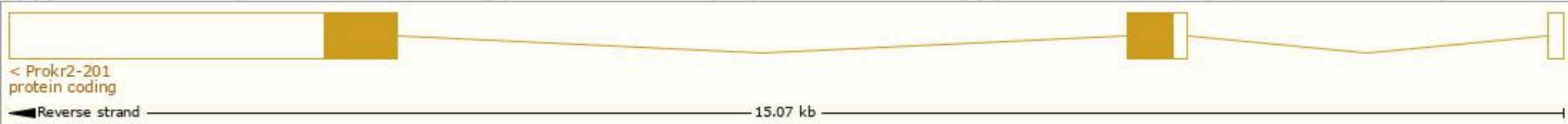
Official Symbol	Prokr2 provided by MGI
Official Full Name	prokineticin receptor 2 provided by MGI
Primary source	MGI:MGI:2181363
See related	Ensembl:ENSMUSG00000050558
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	PKR2; Gpr73l1; Gpcr73l1; EG-VEGRF2; B830005M06Rik
Expression	Biased expression in frontal lobe adult (RPKM 1.4), CNS E18 (RPKM 1.3) and 7 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

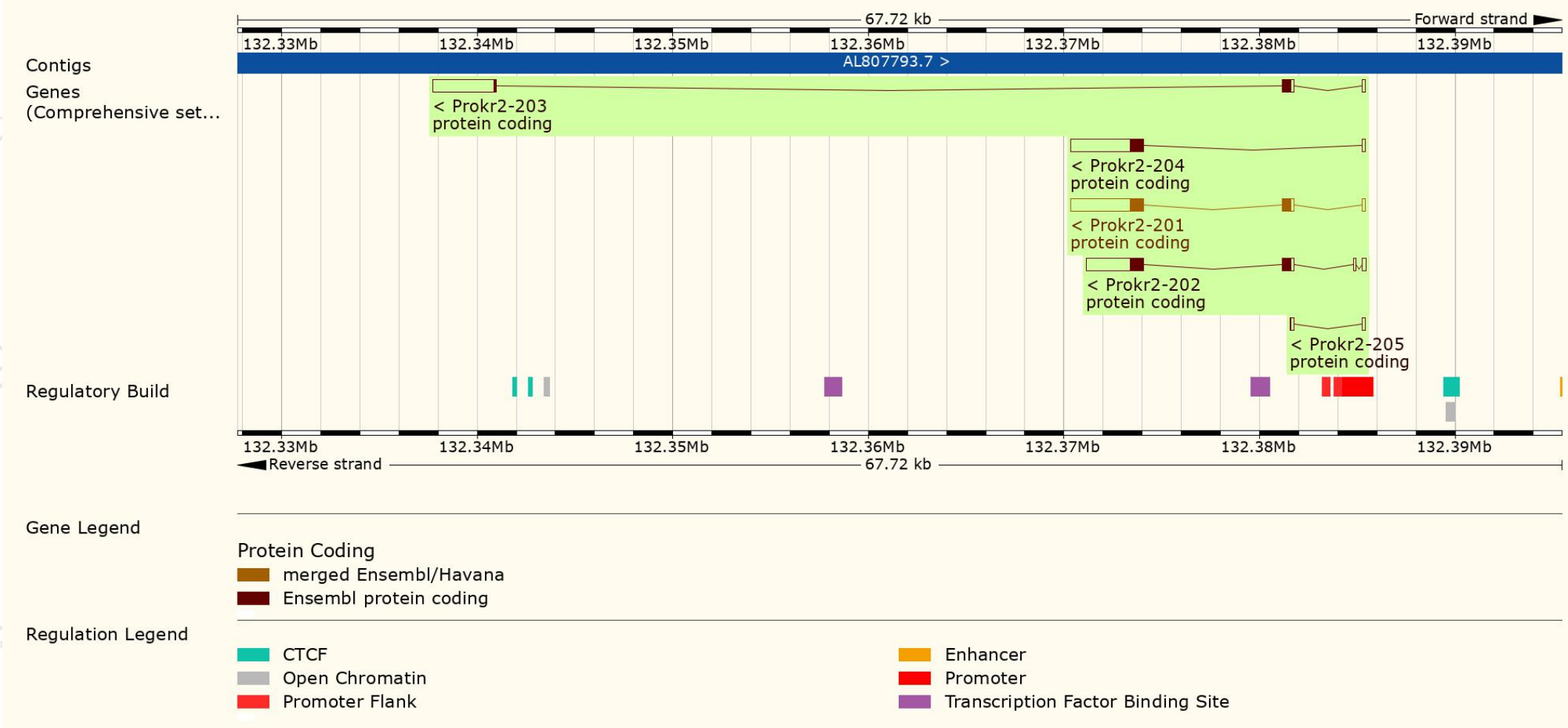
The gene has 5 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Prokr2-201	ENSMUST00000049997.13	4486	381aa	Protein coding	CCDS16773	Q8K458	TSL:1 GENCODE basic APPRIS P1
Prokr2-202	ENSMUST00000110156.1	3829	381aa	Protein coding	CCDS16773	Q8K458	TSL:5 GENCODE basic APPRIS P1
Prokr2-203	ENSMUST00000110157.8	4005	198aa	Protein coding	-	A2AMQ7	TSL:1 GENCODE basic
Prokr2-204	ENSMUST00000142766.1	3932	220aa	Protein coding	-	E0CY28	TSL:1 GENCODE basic
Prokr2-205	ENSMUST00000145995.1	345	17aa	Protein coding	-	A2AMQ9	CDS 3' incomplete TSL:3

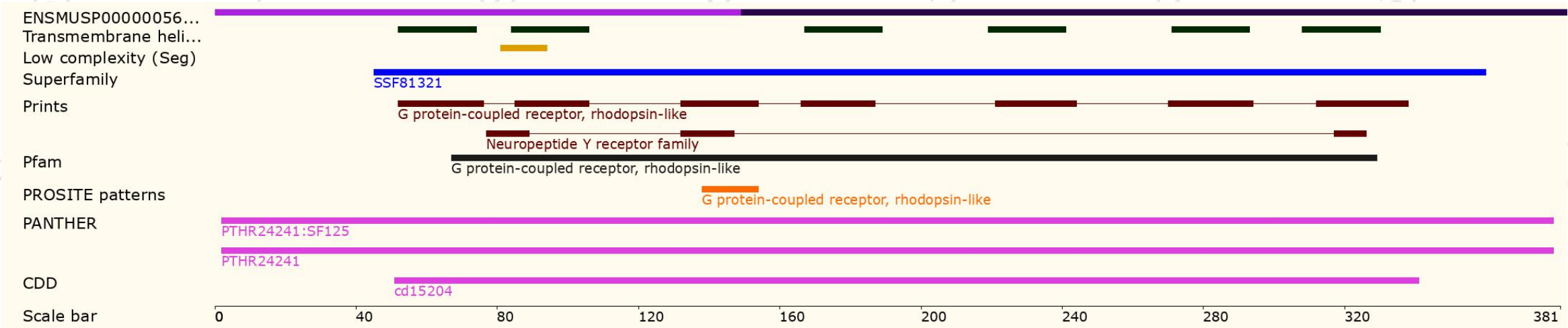
The strategy is based on the design of *Prokr2-201* transcript, The transcription is shown below



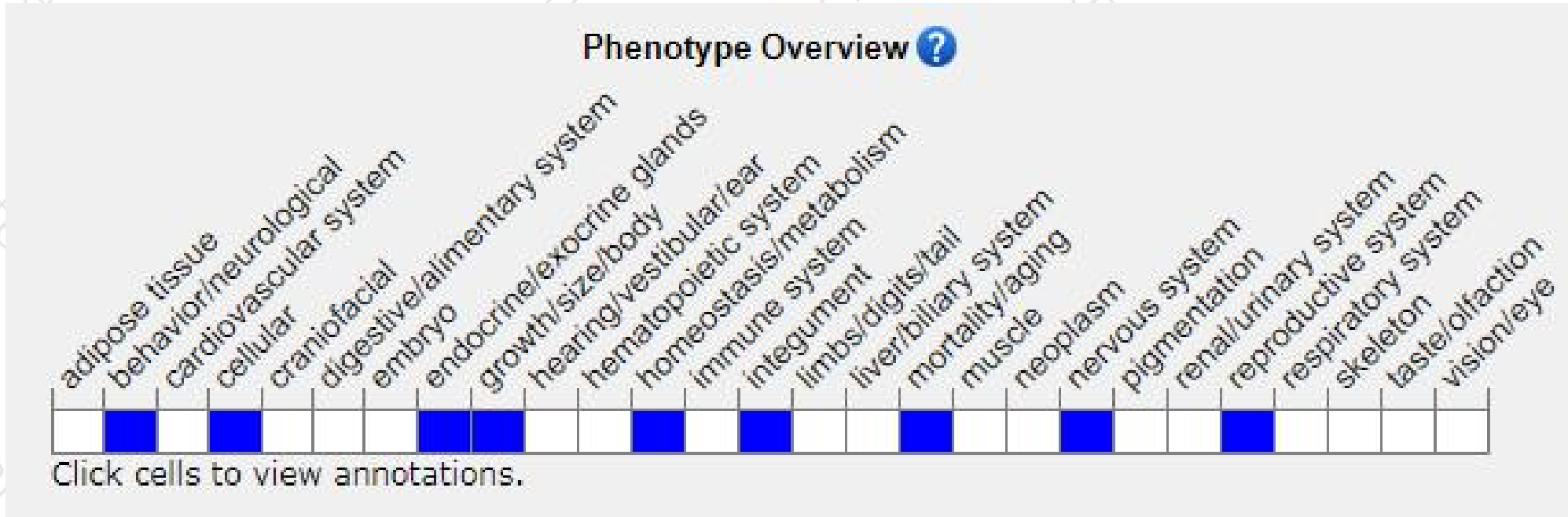
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/marker/MGI:2181363>) .

Homozygotes for a null allele show 50% neonatal lethality, olfactory bulb malformation, and reproductive system atrophy related to a lack of hypothalamic gonadotropin-releasing hormone synthesizing neurons. Homozygotes for another null allele show impaired circadian behavior and thermoregulation.

Existing Model Reporting

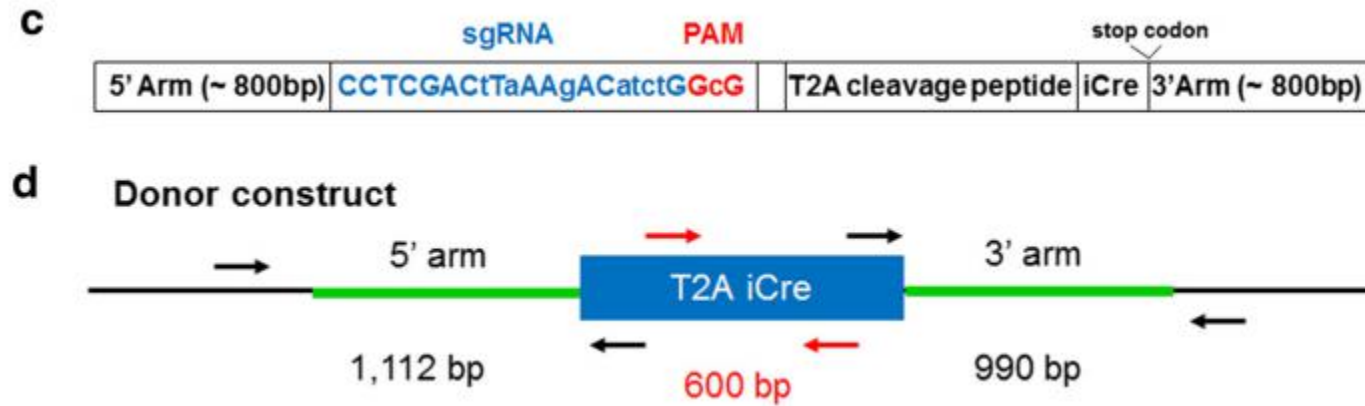


Fig. 1 Generation of the Prokr2-Cre mouse line. **a, b** Chromatogram of DNA sequence from PCR products obtained by amplification across the Cas9 target shows the presence of indels in the genomic DNA due to non-homologous end joining repair of double-strand chromosome breaks induced by sgRNA/Cas9 complexes (**b**). **c, d** Schematic diagrams of the Prokr2-Cre donor construct used in the generation of Prokr2-Cre mouse model (**c**) showing the position of primers used to identify Prokr2-Cre founders (**d**). Note the position of the primers outside the homology arms to control for the correct insertion of Cre into the genome. **e, f** Images of agarose gels showing

[1]Mohsen Z; Sim H; Garcia-Galiano D; Han X; Bellefontaine N; Saunders TL; Elias CF. 2017. Sexually dimorphic distribution of Prokr2 neurons revealed by the Prokr2-Cre mouse model. Brain Struct Funct 222(9):4111-4129.

If you have any questions, you are welcome to inquire.
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